

2/10/04

10/536636
JC-3 Rec'd PCT/PTO 27 MAY 2005
PCT/GB2003/005163

1 **Specific Binding Members and Uses Thereof"**

2

3 The present invention relates to specific binding
4 members and their use in therapy. In particular, the
5 invention relates to specific binding members which
6 bind to CD55, their use in the modulation of
7 complement activation and the treatment of disease,
8 for example, neoplastic disease.

9

10 The human complement system consists of a highly
11 efficient recognition and effector mechanism that
12 consists of 30 serum or cellular components
13 including activated proteins, receptors and positive
14 and negative regulators. In brief, the complement
15 cascade consists of a triggering step, an
16 amplification step with a feedback loop and finally,
17 a membrane attack or lytic step. The central
18 component of the complement system is C3. Generation
19 of C3b by the classical or alternative pathway is
20 crucial for opsonisation and lysis. The classical
21 pathway is initiated when component C1 via its C1q
22 subcomponent attaches to an antibody to form an

1 immune complex. For the alternative pathway,
2 however, there is no initiating factor equivalent to
3 antibody. Rather it is in a state of continuous,
4 low level activation as a result of spontaneous
5 hydrolysis of a thioester group in native C3. This
6 results in binding of C3 to non-specific acceptor
7 molecules in plasma or on cell surfaces. This can
8 result in the formation of C3 convertases and
9 creation of a feedback loop. Because of its potent
10 pro-inflammatory and destructive capabilities, there
11 is a regulatory system designed to prevent
12 complement activation both in the fluid phase and on
13 bystander tissues.

14

15 There are four membrane bound complement regulatory
16 proteins namely complement receptor 1 (CR1), CD55,
17 CD46 and CD59 (Liszewski et al 1996. Adv Immunol
18 61:201-283). Regulation is either accomplished by:

19

- 20 1. Spontaneous decay of activated proteins and
21 enzyme complex (i.e. short half life)
- 22 2. Destabilisation and inhibition of activation
23 complexes
- 24 3. Proteolytic cleavage of "activated" components.

25

26 CD46, CD55 and CD59 are widely expressed on many
27 tissues, including surface epithelia and tumour
28 tissues. In contrast, CR1 expression is limited to
29 peripheral blood cells and is therefore not directly
30 involved in protection of solid tumours.

31

1 Most tumours are of epithelial origin and, although
2 most surface epithelia express complement regulatory
3 proteins, tumours show variable expression of CD55,
4 CD46 and CD59. The majority of colorectal and
5 thyroid cancers express high levels of all three
6 complement regulatory proteins (Niehans et al., 1996
7 Am J Pathol 149:129-142; Li et al., 2001 Br. J.
8 Cancer 84:80-86; Thorsteinsson, 1998 APMIS 106:869-
9 878; Yamakawa et al., 1994 Cancer 73:2808-2817).
10 Ductal carcinoma of the breast shows the most
11 variation in phenotype with some tumours expressing
12 only one inhibitor while others express different
13 combinations of two or three inhibitors (Niehans et
14 al., 1996 supra; Thorsteinsson et al., 1998 supra).
15 Renal cell carcinoma has weak to moderate expression
16 of one to three inhibitors, generally CD55 and CD59
17 (Niehans et al., 1996 supra) whereas non-small cell
18 lung carcinomas and ovarian and cervical cancers
19 usually express CD59 and CD46 with variable CD55
20 immunoreactivity (Niehans et al., 1996 supra; Bjorge
21 et al., 1977 Cancer Immunol Immunother 42:185-192;
22 Simpson et al., 1997 Am J Pathol 151:1455-1467).
23 Similar results have been obtained with established
24 cell lines (Bjorge et al., 1996 supra; Gorter et al
25 1986 Lab Invest 74 1; Juhl et al., 1997 J. Surgical
26 Oncol. 64:222-230; Li et al., 2001 supra).
27
28 All three complement regulatory proteins are
29 expressed on vascular endothelium. Their specific
30 roles during inflammation when the risk of
31 complement mediate injury may be increased remains
32 to be determined. CD55, but not CD46 or CD59, is

1 up-regulated on endothelial cells by the pro-
2 inflammatory mediators $\text{TNF}\alpha$, $\text{IL-1}\beta$, and $\text{IFN-}\gamma$, and
3 also by the MAC (membrane attack complex) and
4 thrombin. These results suggest that CD55 is of
5 critical importance in protecting endothelial cells
6 from complement during inflammation and coagulation.
7 Furthermore it has recently been shown that
8 retraction of endothelial cells exposing sub-
9 endothelial extracellular matrix is a potent inducer
10 of the alternative complement pathway releasing
11 anaphylatoxins that stimulate inflammation. As
12 tumours frequently have dysregulated endothelium,
13 with exposed vessel walls, the tumour environment
14 may induce complement activation. This may be one
15 of the reasons that tumour cells over-express
16 complement regulatory receptors. However, it has
17 been shown that both tumour cells and endothelial
18 cells can actually secrete CD55 but not CD46 into
19 their extracellular matrix (ECM) (Hindmarsh and
20 Marks, 1998 J. Immunol. 160:6128-6136). Hindmarsh
21 and Marks showed that tumour but not endothelial
22 derived CD55 is functionally active and can prevent
23 deposition of C3b. However, deposition of matrix
24 CD55 could not be up-regulated by inflammatory
25 cytokines. More recently the present inventors have
26 shown that both CD55 and CD59 can be deposited into
27 extracellular matrix by both tumours and endothelial
28 cells and the latter can be considerably up-
29 regulated by the potent angiogenesis growth factor
30 VEGF (Li et al., 2001 supra). Furthermore, CD55
31 deposited by endothelial cells stimulated with VEGF
32 was shown to be functionally active. VEGF is

1 unusual, as it is the only cytokine identified to
2 date that up-regulates both cell surface expression
3 and deposition of CD55 into the ECM.

4
5 As most tumours secrete high levels of VEGF to
6 induce angiogenesis they will stimulate expression
7 of CD55 on endothelial cells and within ECM.
8 Interestingly immunohistochemistry of colorectal
9 tumours with anti-CD55 monoclonal antibodies shows
10 intense staining of tumour stroma (Li et al., 2001
11 *supra*; Simpson et al., 1997 *supra*; Niehans et al.,
12 1996 *supra*) and blood vessels (Niehans et al., 1996
13 *supra*). CD55 deposited within ECM is covalently
14 bound as it cannot be released by strong acids or
15 alkalis.

16
17 CD55 binds C3 convertases from both the classical
18 and alternative complement pathways displacing C2b
19 and C3b respectively. It can, therefore, prevent
20 C3b deposition and inhibit the downstream assembly
21 of the membrane attack complex. CD55 has an
22 extracellular domain that is composed of 4
23 contiguous short consensus (SCR) domains and a
24 threonine/serine rich region proximal to the cell
25 surface. It has a single N-glycosylation site
26 between the first and second SCR domains and is
27 heavily O-glycosylated in the threonine and serine
28 rich regions. It is attached to the cell membrane
29 by a glycosphosphoinositol (GPI) anchor and is
30 expressed by all cells exposed to complement,
31 namely, red blood cells, leukocytes, endothelial and
32 epithelial cells. CD55 has also been detected in

1 low amounts in plasma, saliva and urine. The
2 biological significance of this soluble form remains
3 unclear as it has never been shown to be
4 functionally active. Recently it has been shown
5 that HeLa cells and HUVEC incorporate CD55 into
6 their extracellular matrix and that this covalently
7 linked CD55 can inhibit C3b deposition and the
8 release of the pro-inflammatory anaphylatoxin C3a
9 (Hindmarsh and Marks, 1998 *supra*).

10

11 As well as making tumour cells susceptible to *in*
12 *situ* complement activation, antibodies inhibiting
13 the functions of complement regulatory proteins may
14 also make tumour cells susceptible to monoclonal
15 antibody mediated complement dependent cellular
16 cytotoxicity. A chimeric anti-LewisY monoclonal
17 antibody (cH18A) mediated modest complement mediated
18 cell lysis of two lung adenocarcinomas cell lines.
19 However addition of antibodies that block the
20 function of CD46, CD55 and CD59 considerably enhance
21 complement mediated lysis. Use of multiple blocking
22 antibodies to the complement regulatory proteins
23 produced more enhancement of cH18A mediated lysis
24 than any single antibody (Azuma *et al.*, 1995. *Scand*
25 *J Immunol* 42:202-208). Several groups have generated
26 bispecific antibodies with one arm targeting a
27 tumour cell surface antigen and the other targeting
28 the functional domain of a complement regulatory
29 protein. A bispecific antibody targeting HLA and
30 SCR3 of CD55 resulted in a 92% enhancement of C3b
31 deposition on renal tumours. Similarly in the same
32 study a bispecific antibody targeting a renal tumour

1 antigen and the SCR3 of CD55 resulted in a 25-400%
2 increase in C3b deposition on renal tumours and
3 rendered the cells susceptible to complement
4 mediated lysis (Blok et al., 1998 J Immunol
5 160:3437-3443). Finally when a chimeric anti-CD37
6 monoclonal antibody was used to activate the
7 classical complement pathway, a bispecific Fab'gamma
8 construct targeting a lymphoma specific antigen and
9 the CD59 functional domain increased cell lysis by
10 3-5 fold (Harris et al., 1997 Clin. Exp. Immunol.
11 107:364-371).

12

13 However, although previous studies have shown that
14 monoclonal antibodies recognising SCR3 of CD55 could
15 partially neutralise CD55 leading to enhanced C3b
16 deposition and assembly of the MAC complex, each of
17 these antibodies merely compete for binding to SCR3
18 with the C3 convertases and therefore only partially
19 neutralise CD55. Molecular constructs of CD55 have
20 shown that SCR3 is the active domain of CD55 and
21 that SCR2 and SCR4 are necessary to provide the
22 correct conformation for C3 binding. No role for
23 SCR1 in complement decay has been shown. However,
24 although SCR2 is necessary to provide the correct
25 conformation for C3 binding, studies with monoclonal
26 antibodies to single SCR domains of CD55 have shown
27 that only monoclonal antibodies that bind to SCR3
28 and not antibodies that bind to either SCR1 or SCR2
29 can neutralise CD55 (Coyne et al, 1992 J Immunol
30 149, 2906).

31

1 Imaging studies with the monoclonal antibody 791T/36
2 (Embleton et al 1981 Br.J. Cancer 43:582-587) in
3 osteosarcomas, ovarian and colorectal tumours
4 successfully imaged lesions as small as 1cm³
5 (Farrands et al 1982 Lancet 2:397-400; Farrands et
6 al 1983. J. of Bone and Joint Surg. 65:638-640;
7 Armitage et al., 1985. Nucl Med Commun 6:623-631).
8 Furthermore autoradiography of the resected tumours
9 showed both cell surface and intense stromal
10 localisation of the antibody (Armitage et al., 1984
11 Br J Surg 71:407-412). These studies illustrate that
12 an anti-CD55 antibody can effectively localise in
13 tumours without showing any normal tissue toxicity.
14 In particular no detectable binding of radiolabeled
15 antibody to blood cells and only background levels
16 of radiolabel were seen on endothelium or normal
17 tissues. The antigen recognised by 791T/36 was
18 recently identified as CD55 (Spendlove et al Eur J
19 Immunol. 30:2944-2953; Spendlove et al Cancer Res.
20 59:2282-2286). Using CD55/CD46 chimeric constructs
21 it was possible to map the binding site of 791T/36
22 to the first two SCR domains of CD55 with peptide
23 analysis showing that 791T/36 can bind to three
24 distinct regions of SCR1-2 of CD55. One region is in
25 SCR1 and two are in SCR2.
26
27 WO00/5204 discloses a method for making antibodies,
28 for example antibodies directed against decay
29 accelerating factor (DAF, using a naïve antibody
30 phage library. Although the document refers to the
31 use of such antibodies in cancer diagnosis or
32 therapy, no examples are provided other than a

1 speculative example, in which antibody LU30 is
2 suggested for use in assessing overexpression of DAF
3 and for treatment of lung cancer particularly when
4 combined with cytotoxic agents.

5

6 WO/04415 describes the production of the anti-
7 idiotype antibody 105AD7 which was raised against
8 antibody 791T/36 and speculates on potential
9 therapeutic uses of the 105AD7 antibody.

10

11 However, to date, no therapeutically useful anti-
12 CD55 antibodies other than anti SCR3 antibodies have
13 been demonstrated. Therapeutic studies with
14 antibodies directed to other SCRs of this molecule
15 have been limited to immunoconjugated molecules.

16 (See for example US 4916213 (Xoma Corporation), US
17 4925922 (Xoma Corporation) and Byers et al. 1987
18 Cancer Res 47:5042-5046). For example, Byers et al
19 describes studies with 791T/36 linked to ricin A
20 chain, showed significantly inhibition of tumour
21 growth in athymic mice. 791T/36-RTA was therefore
22 screened in a phase I clinical trial in advanced
23 colorectal cancer patients (Byers et al 1989. Cancer
24 Research 49:6153-6160). However the trial was
25 unsuccessful due to dose limiting toxicity.

26

27 Surprisingly, the present inventors have now
28 demonstrated that, although previous studies have
29 demonstrated that antibodies which target either SCR
30 1 or SCR 2 of CD55 failed to have any neutralisation
31 effect on CD55, an antibody which targets both SCR 1

1 and SCR2 not only effectively neutralises CD55 but
2 is superior to a SCR3 neutralising antibody.

3

4 Accordingly, in a first aspect, the present
5 invention provides a method of neutralisation of
6 CD55, comprising administration of a naked binding
7 member which specifically binds to SCR1 and SCR2 of
8 CD55.

9

10 By neutralising CD55, enhanced complement deposition
11 may be facilitated. Accordingly, in a second aspect,
12 the invention provides a method of enhancing
13 complement deposition on a tissue comprising
14 administration of a naked binding member which
15 specifically binds to SCR1 and SCR2 of CD55.

16

17 The methods of the invention may be used *in vitro* or
18 *in vivo*.

19

20 As described above, CD55 is commonly found on many
21 tumour cell surfaces, where it serves to inhibit
22 complement deposition. By neutralising such
23 molecules on tumour cells, the methods of the
24 invention enable complement mediated attack of
25 tumour cells. Accordingly, in a further aspect of
26 the present invention, there is provided a method of
27 treating cancer comprising administration of a
28 therapeutically effective amount of a naked binding
29 member which specifically binds to SCR1 and SCR2 of
30 CD55 to a mammal in need thereof.

31

1 In a further aspect, there is provided the use of
2 (i) a naked binding member which binds to both SCR1
3 and SCR2 of CD55 or (ii) a nucleic acid encoding
4 said binding member in the preparation of a
5 medicament for the neutralisation of CD55.

6

7 In a further aspect, there is provided a naked
8 binding member which binds to both SCR1 and SCR2 for
9 use in the treatment of cancer.

10

11 In a further aspect, there is provided the use of
12 (i) a naked binding member which binds to both SCR1
13 and SCR2 of CD55 or (ii) a nucleic acid encoding
14 said binding member in the preparation of a
15 medicament for treating cancer.

16

17 The present invention also provides a pharmaceutical
18 composition for the treatment of cancer, wherein the
19 composition comprises a naked binding member that
20 binds to both SCR1 and SCR2 of CD55.

21

22 **Specific Binding Member**

23

24 As used herein, a "binding member" is a member of a
25 pair of molecules which have binding specificity for
26 one another. The binding member is, therefore, a
27 specific binding member. The members of a binding
28 pair may be naturally derived or wholly or partially
29 synthetically produced. One member of the pair of
30 molecules may have an area on its surface, which may
31 be a protrusion or a cavity, which specifically
32 binds to and is therefore complementary to a

1 particular spatial and polar organisation of the
2 other member of the pair of molecules. Thus, the
3 members of the pair have the property of binding
4 specifically to each other. Examples of types of
5 binding pairs are antigen-antibody, biotin-avidin,
6 hormone-hormone receptor, receptor-ligand, enzyme-
7 substrate. The present invention is concerned with
8 antigen-antibody type reactions, although a binding
9 member of the invention and for use in the invention
10 may be any moiety which can bind to both SCR1 and
11 SCR2 of CD55.

12

13 As used herein, "naked" means that the binding
14 member of or for use in the present invention is not
15 bound to, for example conjugated with, any agent,
16 for example ricin, having anti-tumour properties.

17

18 **Antibodies**

19

20 An "antibody" is an immunoglobulin, whether natural
21 or partly or wholly synthetically produced. The
22 term also covers any polypeptide, protein or peptide
23 having a binding domain which is, or is homologous
24 to, an antibody binding domain. These can be
25 derived from natural sources, or they may be partly
26 or wholly synthetically produced. Examples of
27 antibodies are the immunoglobulin isotypes and their
28 isotypic subclasses and fragments which comprise an
29 antigen binding domain such as Fab, scFv, Fv, dAb,
30 Fd; and diabodies.

31

1 The binding member of the invention may be an
2 antibody such as a monoclonal or polyclonal
3 antibody, or a fragment thereof. The constant region
4 of the antibody may be of any class including, but
5 not limited to, human classes IgG, IgA, IgM, IgD and
6 IgE. The antibody may belong to any sub class e.g.
7 IgG1, IgG2, IgG3 and IgG4. IgG1 is preferred. In
8 preferred embodiments the antibody is 791T/36
9 produced by the cell line deposited with ATCC under
10 accession no. HB9173.

11

12 As antibodies can be modified in a number of ways,
13 the term "antibody" should be construed as covering
14 any binding member or substance having a binding
15 domain with the required specificity. Thus, this
16 term covers antibody fragments, derivatives,
17 functional equivalents and homologues of antibodies,
18 including any polypeptide comprising an
19 immunoglobulin binding domain, whether natural or
20 wholly or partially synthetic. Chimeric molecules
21 comprising an immunoglobulin binding domain, or
22 equivalent, fused to another polypeptide are
23 therefore included. Cloning and expression of
24 chimeric antibodies are described in EP-A-0120694
25 and EP-A-0125023.

26

27 It has been shown that fragments of a whole antibody
28 can perform the function of binding antigens.
29 Examples of such binding fragments are (i) the Fab
30 fragment consisting of VL, VH, CL and CH1 domains;
31 (ii) the Fd fragment consisting of the VH and CH1
32 domains; (iii) the Fv fragment consisting of the VL

1 and VH domains of a single antibody; (iv) the dAb
2 fragment (Ward, E.S. et al., *Nature* 341:544-546
3 (1989)) which consists of a VH domain; (v) isolated
4 CDR regions; (vi) F(ab')₂ fragments, a bivalent
5 fragment comprising two linked Fab fragments (vii)
6 single chain Fv molecules (scFv), wherein a VH
7 domain and a VL domain are linked by a peptide
8 linker which allows the two domains to associate to
9 form an antigen binding site (Bird et al., *Science*
10 242:423-426 (1988); Huston et al., *PNAS USA* 85:5879-
11 5883 (1988)); (viii) bispecific single chain Fv
12 dimers (PCT/US92/09965) and (ix) "diabodies",
13 multivalent or multispecific fragments constructed
14 by gene fusion (WO94/13804; P. Hollinger et al.,
15 *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993)).

16

17 A fragment of an antibody or of a polypeptide for
18 use in the present invention, for example, a
19 fragment of the 791T/36 antibody, generally means a
20 stretch of amino acid residues of at least 5 to 7
21 contiguous amino acids, often at least about 7 to 9
22 contiguous amino acids, typically at least about 9
23 to 13 contiguous amino acids, more preferably at
24 least about 20 to 30 or more contiguous amino acids
25 and most preferably at least about 30 to 40 or more
26 consecutive amino acids. A preferred group of
27 fragments are those which include all or part of the
28 CDR regions of monoclonal antibody 791T/36. A
29 preferred group of fragments are those which include
30 all or part of the CDR regions of monoclonal
31 antibody 791T/36.

32

1 A "derivative" of such an antibody or polypeptide,
2 or of a fragment of a 791T/36 antibody means an
3 antibody or polypeptide modified by varying the
4 amino acid sequence of the protein, e.g. by
5 manipulation of the nucleic acid encoding the
6 protein or by altering the protein itself. Such
7 derivatives of the natural amino acid sequence may
8 involve insertion, addition, deletion and/or
9 substitution of one or more amino acids, preferably
10 while providing a peptide having anti-CD55 activity,
11 for example, CD55 neutralisation activity.
12 Preferably such derivatives involve the insertion,
13 addition, deletion and/or substitution of 25 or
14 fewer amino acids, more preferably of 15 or fewer,
15 even more preferably of 10 or fewer, more preferably
16 still of 4 or fewer and most preferably of 1 or 2
17 amino acids only.

18

19 The term "antibody" includes antibodies which have
20 been "humanised". Methods for making humanised
21 antibodies are known in the art. Methods are
22 described, for example, in Winter, U.S. Patent No.
23 5,225,539. A humanised antibody may be a modified
24 antibody having the hypervariable region of a
25 monoclonal antibody such as 791T/36 and the constant
26 region of a human antibody. Thus the binding member
27 may comprise a human constant region.

28

29 The variable region other than the hypervariable
30 region may also be derived from the variable region
31 of a human antibody and/or may also be derived from
32 a monoclonal antibody such as 791T/36. In such

1 case, the entire variable region may be derived from
2 murine monoclonal antibody 791T/36 and the antibody
3 is said to be chimerised. Methods for making
4 chimerised antibodies are known in the art. Such
5 methods include, for example, those described in
6 U.S. patents by Boss (Celltech) and by Cabilly
7 (Genentech). See U.S. Patent Nos. 4,816,397 and
8 4,816,567, respectively.

9
10 It is possible to take monoclonal and other
11 antibodies and use techniques of recombinant DNA
12 technology to produce other antibodies or chimeric
13 molecules which retain the specificity of the
14 original antibody. Such techniques may involve
15 introducing DNA encoding the immunoglobulin variable
16 region, or the complementary determining regions
17 (CDRs), of an antibody to the constant regions, or
18 constant regions plus framework regions, of a
19 different immunoglobulin. See, for instance, EP-A-
20 184187, GB 2188638A or EP-A-239400. A hybridoma or
21 other cell producing an antibody may be subject to
22 genetic mutation or other changes, which may or may
23 not alter the binding specificity of antibodies
24 produced.

25
26 In preferred embodiments of the invention, the
27 binding member binds to CD55 SCR1 (amino acids 83-
28 93) and SCR2 (amino acids 101-112 and amino acids
29 145-157) of the sequences shown in Figure 1b.

30

31 The binding member may comprise one or more of the
32 CDRs of the antibody, or a fragment thereof,

1 produced by the cell line deposited at ATCC under
2 accession number HB9173.

3
4 As described above, in a preferred embodiment of the
5 invention, the binding member is the antibody
6 791T/36 produced by the hybridoma cell deposited
7 under ATCC accession number HB9173. As used herein,
8 reference to "791T/36" includes sequences which show
9 substantial homology with 791T/36. Preferably the
10 degree of homology between 791T/36 complementary
11 determining regions (CDRs) and the CDRs of other
12 antibodies will be at least 60%, more preferably
13 70%, further preferably 80%, even more preferably
14 90% or most preferably 95%.

15
16 The percent identity of two amino acid sequences or
17 of two nucleic acid sequences may be determined by
18 aligning the sequences for optimal comparison
19 purposes (e.g., gaps can be introduced in the first
20 sequence for best alignment with the sequence) and
21 comparing the amino acid residues or nucleotides at
22 corresponding positions. The "best alignment" is an
23 alignment of two sequences which results in the
24 highest percent identity. The percent identity is
25 determined by the number of identical amino acid
26 residues or nucleotides in the sequences being
27 compared (i.e., % identity = number of identical
28 positions/total number of positions x 100).

29
30 The determination of percent identity between two
31 sequences can be accomplished using a mathematical
32 algorithm known to those of skill in the art. An

1 example of a mathematical algorithm for comparing
2 two sequences is the algorithm of Karlin and
3 Altschul (1990) *Proc. Natl. Acad. Sci. USA*
4 87:2264-2268, modified as in Karlin and Altschul
5 (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. The
6 NBLAST and XBLAST programs of Altschul, et al.
7 (1990) *J. Mol. Biol.* 215:403-410 have incorporated
8 such an algorithm. BLAST nucleotide searches can be
9 performed with the NBLAST program, score = 100,
10 wordlength = 12 to obtain nucleotide sequences
11 homologous to nucleic acid molecules of the
12 invention. BLAST protein searches can be performed
13 with the XBLAST program, score = 50, wordlength = 3
14 to obtain amino acid sequences homologous to protein
15 molecules of the invention. To obtain gapped
16 alignments for comparison purposes, Gapped BLAST can
17 be utilised as described in Altschul et al. (1997)
18 *Nucleic Acids Res.* 25:3389-3402. Alternatively,
19 PSI-Blast can be used to perform an iterated search
20 which detects distant relationships between
21 molecules (*Id.*). When utilising BLAST, Gapped
22 BLAST, and PSI-Blast programs, the default
23 parameters of the respective programs (e.g., XBLAST
24 and NBLAST) can be used. See
25 <http://www.ncbi.nlm.nih.gov>.

26
27 Another example of a mathematical algorithm utilised
28 for the comparison of sequences is the algorithm of
29 Myers & Miller, CABIOS (1989). The ALIGN program
30 (version 2.0) which is part of the CGC sequence
31 alignment software package has incorporated such an
32 algorithm. Other algorithms for sequence analysis

1 known in the art include ADVANCE and ADAM as
2 described in Torellis & Robotti (1994) *Comput. Appl.*
3 *Biosci.*, 10 :3-5; and FASTA described in Pearson &
4 Lipman (1988) *Proc. Natl. Acad. Sci.* 85:2444-8.
5 Within FASTA, ktup is a control option that sets the
6 sensitivity and speed of the search.

7
8 Where high degrees of sequence identity are present
9 there will be relatively few differences in amino
10 acid sequence. Thus for example they may be less
11 than 20, less than 10, or even less than 5
12 differences.

13
14 The present inventors have shown that antibodies
15 directed to SCR1 and SCR2 of CD55, for example
16 791T/36 antibodies and fragments and derivatives
17 thereof can be used as cancer therapeutics to
18 inactivate CD55 and make tumour cells susceptible to
19 complement mediated attack. This is exemplified by
20 localisation of the antibody within tumours of
21 cancer patients and their subsequent enhanced
22 survival (see the Examples). Accordingly the
23 invention further provides the use of naked
24 "fragments" or "derivatives" of 791T/36 or other
25 polypeptides of the "791T/36" family which bind to
26 both SCR1 and SCR2 CD55 epitopes in the preparation
27 of an agent for treating cancer.

28
29 The binding members may be administered alone or in
30 combination with one or more further agents. Thus,
31 the present invention further provides products
32 comprising a naked binding member, which binds to

1 both SCR1 and SCR2 of CD55, and an active agent as a
2 combined preparation for simultaneous, separate or
3 sequential use in the treatment of cancer. Active
4 agents may include chemotherapeutic agents
5 including, Doxorubicin, taxol, 5-Fluorouracil (5
6 FU), Leucovorin, Irinotecan, Mitomycin C,
7 Oxaliplatin, Raltitrexed, Tamoxifen and Cisplatin
8 which may operate synergistically with the binding
9 member of the present invention. Other active agents
10 may include suitable doses of pain relief drugs such
11 as non-steroidal anti-inflammatory drugs (e.g.
12 aspirin, paracetamol, ibuprofen or ketoprofen) or
13 opiates such as morphine, or anti-emetics. In
14 further embodiments, the active agent may be a
15 further binding member. Thus, in preferred
16 embodiments the binding member may be administered
17 in combination with one or more further binding
18 members. Such binding members may include but are
19 not limited to an anti-CD20 antibody e.g. Rituxan
20 (Rituximab) (Biogen IDEC (Cambridge, MA, USA); an
21 anti-VEGF antibody e.g. Avastin (bevacizumab),
22 Genentech (South San Francisco, CA, USA) / Roche
23 (Basel, Switzerland); an anti-CD171A antibody, e.g.
24 Panorex (edrecolomab) Centocor (Malvern, PA, USA) /
25 Glaxo SmithKline (Uxbridge, UK); an anti-CEA anti-
26 idiotypic mAb e.g. CeaVac, Titan Pharmaceuticals
27 (South San Francisco, CA, USA); an anti-EGFR
28 antibody e.g. Erbitux (cetuximab), ImClone (New York,
29 USA) / Bristol Myers Squibb (New York, USA), Merck
30 (Whitehouse Station, NJ, USA); an anti-HMFG anti-
31 idiotypic mAb e.g. TriAb, Titan Pharmaceuticals
32 (South San Francisco, CA, USA), an anti-EGFR

1 antibody e.g. ABX-EGF, Abgenix (Fremont, CA, USA)
2 /Amgen Thousand Oaks, CA) and/or an anti-HER2
3 antibody e.g. Herceptin, Genentech (South San
4 Francisco, CA, USA).

5
6 Preferably, the active agent synergises with the
7 binding member. The ability of the binding member to
8 synergise with an active agent to enhance tumour
9 killing may not be due to immune effector mechanisms
10 but rather may be a direct consequence of
11 inactivating CD55 allowing enhanced complement
12 deposition and complement lysis. The binding member
13 of the invention may carry a detectable label.

14

15 **Treatment**

16

17 "Treatment" includes any regime that can benefit a
18 human or non-human animal. The treatment may be in
19 respect of an existing condition or may be
20 prophylactic (preventative treatment). Treatment may
21 include curative, alleviation or prophylactic
22 effects.

23

24 "Treatment of cancer" includes treatment of
25 conditions caused by cancerous growth and includes
26 the treatment of neoplastic growths or tumours.
27 Examples of tumours that can be treated by the
28 system of the invention are, for instance, sarcomas,
29 including osteogenic and soft tissue sarcomas,
30 carcinomas, e.g., breast-, lung-, bladder-, thyroid-
31 , prostate-, colon-, rectum-, pancreas-, stomach-,
32 liver-, uterine-, cervical and ovarian carcinoma,

1 lymphomas, including Hodgkin and non-Hodgkin
2 lymphomas, neuroblastoma, melanoma, myeloma, Wilms
3 tumor, and leukemias, including acute lymphoblastic
4 leukaemia and acute myeloblastic leukaemia, gliomas
5 and retinoblastomas.

6
7 The binding member may, upon binding to SCR1 and
8 SCR2 of CD55 present on cancerous cells or tissues,
9 including tumour and non-tumour cells, neutralise
10 CD55 and enhance complement deposition and
11 complement mediated lysis of these cells.

12
13 The compositions and methods of the invention may be
14 particularly useful in the treatment of existing
15 cancer and in the prevention of the recurrence of
16 cancer after initial treatment or surgery.

17
18 **Administration**

19
20 Binding members of the present invention may be
21 administered alone but will preferably be
22 administered as a pharmaceutical composition, which
23 will generally comprise a suitable pharmaceutical
24 excipient, diluent or carrier selected dependent on
25 the intended route of administration.

26 Binding members of the present invention may be
27 administered to a patient in need of treatment via
28 any suitable route. The precise dose will depend
29 upon a number of factors, including the precise
30 nature of the member (e.g. whole antibody, fragment
31 or diabody), and the nature of the detectable label
32 attached to the member.

1
2 Some suitable routes of administration include (but
3 are not limited to) oral, rectal, nasal, topical
4 (including buccal and sublingual), vaginal or
5 parenteral (including subcutaneous, intramuscular,
6 intravenous, intradermal, intrathecal and epidural)
7 administration. Intravenous administration is
8 preferred.

9
10 It is envisaged that injections (intravenous) will
11 be the primary route for therapeutic administration
12 of the compositions although delivery through a
13 catheter or other surgical tubing is also envisaged.
14 Liquid formulations may be utilised after
15 reconstitution from powder formulations.

16
17 For intravenous, injection, or injection at the site
18 of affliction, the active ingredient will be in the
19 form of a parenterally acceptable aqueous solution
20 which is pyrogen-free and has suitable pH,
21 isotonicity and stability. Those of relevant skill
22 in the art are well able to prepare suitable
23 solutions using, for example, isotonic vehicles such
24 as Sodium Chloride Injection, Ringer's Injection,
25 Lactated Ringer's Injection. Preservatives,
26 stabilisers, buffers, antioxidants and/or other
27 additives may be included, as required.

28
29 Pharmaceutical compositions for oral administration
30 may be in tablet, capsule, powder or liquid form. A
31 tablet may comprise a solid carrier such as gelatin
32 or an adjuvant. Liquid pharmaceutical compositions

1 generally comprise a liquid carrier such as water,
2 petroleum, animal or vegetable oils, mineral oil or
3 synthetic oil. Physiological saline solution,
4 dextrose or other saccharide solution or glycols
5 such as ethylene glycol, propylene glycol or
6 polyethylene glycol may be included.

7
8 The composition may also be administered via
9 microspheres, liposomes, other microparticulate
10 delivery systems or sustained release formulations
11 placed in certain tissues including blood. Suitable
12 examples of sustained release carriers include
13 semipermeable polymer matrices in the form of shared
14 articles, e.g. suppositories or microcapsules.
15 Implantable or microcapsular sustained release
16 matrices include polylactides (US Patent No. 3, 773,
17 919; EP-A-0058481) copolymers of L-glutamic acid and
18 gamma ethyl-L-glutamate (Sidman et al, Biopolymers
19 22(1): 547-556, 1985), poly (2-hydroxyethyl-
20 methacrylate) or ethylene vinyl acetate (Langer et
21 al, J. Biomed. Mater. Res. 15: 167-277, 1981, and
22 Langer, Chem. Tech. 12:98-105, 1982). Liposomes
23 containing the polypeptides are prepared by well-
24 known methods: DE 3,218, 121A; Epstein et al, PNAS
25 USA, 82: 3688-3692, 1985; Hwang et al, PNAS USA, 77:
26 4030-4034, 1980; EP-A-0052522; E-A-0036676; EP-A-
27 0088046; EP-A-0143949; EP-A-0142541; JP-A-83-11808;
28 US Patent Nos 4,485,045 and 4,544,545. Ordinarily,
29 the liposomes are of the small (about 200-800
30 Angstroms) unilamellar type in which the lipid
31 content is greater than about 30 mol. % cholesterol,

1 the selected proportion being adjusted for the
2 optimal rate of the polypeptide leakage.

3

4 Examples of the techniques and protocols mentioned
5 above and other techniques and protocols which may
6 be used in accordance with the invention can be
7 found in Remington's Pharmaceutical Sciences, 16th
8 edition, Oslo, A. (ed), 1980.

9

10 The composition may be administered in a localised
11 manner to a tumour site or other desired site or may
12 be delivered in a manner in which it targets tumour
13 or other cells. Targeting therapies may be used to
14 deliver the active agent more specifically to
15 certain types of cell, by the use of targeting
16 systems such as antibody or cell specific ligands.
17 Targeting may be desirable for a variety of reasons,
18 for example if the agent is unacceptably toxic, or
19 if it would otherwise require too high a dosage, or
20 if it would not otherwise be able to enter the
21 target cells.

22

23 **Pharmaceutical Compositions**

24

25 As described above, the present invention extends to
26 a pharmaceutical composition for the treatment of
27 cancer, the composition comprising a naked binding
28 member which binds to both SCR1 and SCR2 of CD55.

29 Pharmaceutical compositions according to the present
30 invention, and for use in accordance with the
31 present invention may comprise, in addition to
32 active ingredient, a pharmaceutically acceptable

1 excipient, carrier, buffer stabiliser or other
2 materials well known to those skilled in the art.
3 Such materials should be non-toxic and should not
4 interfere with the efficacy of the active
5 ingredient. The precise nature of the carrier or
6 other material will depend on the route of
7 administration, which may be oral, or by injection,
8 e.g. intravenous.

9
10 The formulation may be a liquid, for example, a
11 physiologic salt solution containing non-phosphate
12 buffer at pH 6.8-7.6, or a lyophilised powder.

13

14 **Dose**

15

16 The compositions are preferably administered to an
17 individual in a "therapeutically effective amount",
18 this being sufficient to show benefit to the
19 individual. The actual amount administered, and
20 rate and time-course of administration, will depend
21 on the nature and severity of what is being treated.
22 Prescription of treatment, e.g. decisions on dosage
23 etc, is ultimately within the responsibility and at
24 the discretion of general practitioners and other
25 medical doctors, and typically takes account of the
26 disorder to be treated, the condition of the
27 individual patient, the site of delivery, the method
28 of administration and other factors known to
29 practitioners.

30

31 The optimal dose can be determined by physicians
32 based on a number of parameters including, for

1 example, age, sex, weight, severity of the condition
2 being treated, the active ingredient being
3 administered and the route of administration. In
4 general, a serum concentration of polypeptides and
5 antibodies that permits saturation of receptors is
6 desirable. A concentration in excess of
7 approximately 0.1nM is normally sufficient. For
8 example, a dose of 100mg/m² of antibody provides a
9 serum concentration of approximately 20nM for
10 approximately eight days.

11

12 As a rough guideline, doses of antibodies may be
13 given weekly in amounts of 10-300mg/m². Equivalent
14 doses of antibody fragments should be used at more
15 frequent intervals in order to maintain a serum
16 level in excess of the concentration that permits
17 saturation of CD55.

18

19 **Production of Binding Members**

20

21 The binding members of and for use in the present
22 invention may be generated wholly or partly by
23 chemical synthesis. The binding members can be
24 readily prepared according to well-established,
25 standard liquid or, preferably, solid-phase peptide
26 synthesis methods, general descriptions of which are
27 broadly available (see, for example, in J.M. Stewart
28 and J.D. Young, Solid Phase Peptide Synthesis, 2nd
29 edition, Pierce Chemical Company, Rockford, Illinois
30 (1984), in M. Bodanzsky and A. Bodanzsky, The
31 Practice of Peptide Synthesis, Springer Verlag, New
32 York (1984); and Applied Biosystems 430A Users

1 Manual, ABI Inc., Foster City, California), or they
2 may be prepared in solution, by the liquid phase
3 method or by any combination of solid-phase, liquid
4 phase and solution chemistry, e.g. by first
5 completing the respective peptide portion and then,
6 if desired and appropriate, after removal of any
7 protecting groups being present, by introduction of
8 the residue X by reaction of the respective carbonic
9 or sulfonic acid or a reactive derivative thereof.

10

11 Another convenient way of producing a binding member
12 suitable for use in the present invention is to
13 express nucleic acid encoding it, by use of nucleic
14 acid in an expression system. Thus the present
15 invention further provides the use of an isolated
16 nucleic acid encoding a naked binding member which
17 binds to both SCR1 and SCR2 of CD55 in the
18 preparation of an agent for treating cancer.

19

20 Nucleic acid for use in accordance with the present
21 invention may comprise DNA or RNA and may be wholly
22 or partially synthetic. In a preferred aspect,
23 nucleic acid for use in the invention codes for a
24 binding member of the invention as defined above.
25 The skilled person will be able to determine
26 substitutions, deletions and/or additions to such
27 nucleic acids which will still provide a binding
28 member of the present invention.

29

30 Nucleic acid sequences encoding a binding member for
31 use with the present invention can be readily
32 prepared by the skilled person using the information

1 and references contained herein and techniques known
2 in the art (for example, see Sambrook, Fritsch and
3 Maniatis, "Molecular Cloning", A Laboratory Manual,
4 Cold Spring Harbor Laboratory Press, 1989, and
5 Ausubel et al, Short Protocols in Molecular Biology,
6 John Wiley and Sons, 1992), given the nucleic acid
7 sequences and clones available. These techniques
8 include (i) the use of the polymerase chain reaction
9 (PCR) to amplify samples of such nucleic acid, e.g.
10 from genomic sources, (ii) chemical synthesis, or
11 (iii) preparing cDNA sequences. DNA encoding
12 antibody fragments may be generated and used in any
13 suitable way known to those of skill in the art,
14 including by taking encoding DNA, identifying
15 suitable restriction enzyme recognition sites either
16 side of the portion to be expressed, and cutting out
17 said portion from the DNA. The portion may then be
18 operably linked to a suitable promoter in a standard
19 commercially available expression system. Another
20 recombinant approach is to amplify the relevant
21 portion of the DNA with suitable PCR primers.
22 Modifications to the sequences can be made, e.g.
23 using site directed mutagenesis, to lead to the
24 expression of modified peptide or to take account of
25 codon preferences in the host cells used to express
26 the nucleic acid.

27

28 The nucleic acid may be comprised as constructs in
29 the form of a plasmid, vector, transcription or
30 expression cassette which comprises at least one
31 nucleic acid as described above. The construct may
32 be comprised within a recombinant host cell which

1 comprises one or more constructs as above.
2 Expression may conveniently be achieved by culturing
3 under appropriate conditions recombinant host cells
4 containing the nucleic acid. Following production
5 by expression a specific binding member may be
6 isolated and/or purified using any suitable
7 technique, then used as appropriate.

8
9 Binding members-encoding nucleic acid molecules and
10 vectors for use in accordance with the present
11 invention may be provided isolated and/or purified,
12 e.g. from their natural environment, in
13 substantially pure or homogeneous form, or, in the
14 case of nucleic acid, free or substantially free of
15 nucleic acid or genes origin other than the sequence
16 encoding a polypeptide with the required function.

17
18 Systems for cloning and expression of a polypeptide
19 in a variety of different host cells are well known.
20 Suitable host cells include bacteria, mammalian
21 cells, yeast and baculovirus systems. Mammalian
22 cell lines available in the art for expression of a
23 heterologous polypeptide include Chinese hamster
24 ovary cells, HeLa cells, baby hamster kidney cells,
25 NSO mouse melanoma cells and many others. A common,
26 preferred bacterial host is *E. coli*.

27
28 The expression of antibodies and antibody fragments
29 in prokaryotic cells such as *E. coli* is well
30 established in the art. For a review, see for
31 example Plückthun, *Bio/Technology* 9:545-551 (1991).
32 Expression in eukaryotic cells in culture is also

1 available to those skilled in the art as an option
2 for production of a binding member, see for recent
3 review, for example Reff, *Curr. Opinion Biotech.*
4 4:573-576 (1993); Trill *et al.*, *Curr. Opinion*
5 *Biotech.* 6:553-560 (1995).

6
7 Alternatively, the specific binding members for use
8 in the invention may be produced in transgenic
9 organisms, for example mammals, avians, fish,
10 insects or plants using methods known in the art. In
11 such transgenic methods, nucleic acid encoding the
12 binding member(s) may be introduced to the cell or
13 embryo by methods including but not limited to
14 direct injection, electroporation, nuclear transfer
15 techniques or by use of vectors, e.g. viral vectors.
16 In one preferred embodiment, the specific binding
17 members are produced in avian tissues, preferably
18 avian eggs, using, for example, the method as
19 disclosed in GB 0227645.9, filed 27 November 2002
20 and the subsequent PCT application claiming priority
21 therefrom.

22
23 Suitable vectors can be chosen or constructed,
24 containing appropriate regulatory sequences,
25 including promoter sequences, terminator sequences,
26 polyadenylation sequences, enhancer sequences,
27 marker genes and other sequences as appropriate.
28 Vectors may be plasmids, viral e.g. 'phage, or
29 phagemid, as appropriate. For further details see,
30 for example, Sambrook *et al.*, *Molecular Cloning: A*
31 *Laboratory Manual*: 2nd Edition, Cold Spring Harbor
32 Laboratory Press (1989). Many known techniques and

1 protocols for manipulation of nucleic acid, for
2 example in preparation of nucleic acid constructs,
3 mutagenesis, sequencing, introduction of DNA into
4 cells and gene expression, and analysis of proteins,
5 are described in detail in Ausubel et al. eds.,
6 *Short Protocols in Molecular Biology*, 2nd Edition,
7 John Wiley & Sons (1992).

8
9 The nucleic acid may be introduced into a host cell
10 by any suitable means. The introduction may employ
11 any available technique. For eukaryotic cells,
12 suitable techniques may include calcium phosphate
13 transfection, DEAE-Dextran, electroporation,
14 liposome-mediated transfection and transduction
15 using retrovirus or other virus, e.g. vaccinia or,
16 for insect cells, baculovirus. For bacterial cells,
17 suitable techniques may include calcium chloride
18 transformation, electroporation and transfection
19 using bacteriophage.

20
21 Marker genes such as antibiotic resistance or
22 sensitivity genes may be used in identifying clones
23 containing nucleic acid of interest, as is well
24 known in the art.

25
26 The introduction may be followed by causing or
27 allowing expression from the nucleic acid, e.g. by
28 culturing host cells under conditions for expression
29 of the gene.

30
31 The nucleic acid may be integrated into the genome
32 (e.g. chromosome) of the host cell. Integration may

1 be promoted by inclusion of sequences which promote
2 recombination with the genome in accordance with
3 standard techniques. The nucleic acid may be on an
4 extra-chromosomal vector within the cell, or
5 otherwise identifiably heterologous or foreign to
6 the cell.

7

8 **Assays**

9

10 The invention further provides assays for
11 identification of further agents, for example
12 antibodies that can be used for the enhancement of
13 complement deposition on a cell sample or tissue and
14 which can optionally be used in the treatment of
15 cancer.

16

17 In a preferred aspect, the assay comprises an assay
18 method for identification of an agent capable of
19 inhibiting CD55 comprising steps:

20

- 21 a) bringing into contact a candidate agent with at
22 least a portion of SCR1 and SCR2 of CD55; and
23
24 b) determining binding of said candidate agent to
25 both SCR1 and SCR2.

26

27 In a further embodiment, the assay method comprises
28 a method for identification of an agent capable of
29 inhibiting CD55 comprising:

30

- 31 (a) bringing into contact a candidate agent with at
32 least a portion of SCR1 and SCR2 of CD55 in the

1 presence of a naked binding member which in the
2 absence of the candidate agent is capable of
3 binding both SCR1 and SCR2 of CD55; and

4

5 (b) determining the extent to which the candidate
6 agent inhibits binding of the naked binding
7 member to SCR1 and SCR2 of CD55.

8

9 The assays may further comprise the step of
10 selecting a candidate agent which binds both SCR1
11 and SCR2 of CD55; and/or the step of determining
12 the amount of complement deposition on a cell sample
13 in the presence and absence of the candidate agent.

14

15 In preferred embodiments of the assays of the
16 invention, the portion of SCR1 and SCR2 of CD55
17 comprises amino acids 83-93, 101-112 and 145-157 of
18 the sequences shown in Figure 1b.

19

20 The present invention further provides a screening
21 method comprising the step of screening a library of
22 candidate agents for the ability to inhibit the
23 binding of a naked binding member to both SCR1 and
24 SCR2 of CD55.

25

26 The assay of the invention may be a screen , whereby
27 a number of candidate agents are tested.

28 Accordingly, any suitable technique for screening
29 compounds known to the person skilled in the art may
30 be used. The screen may be a high-throughput
31 screen. For example, WO84/03564 describes a method
32 in which large numbers of peptides are synthesised

1 on a solid substrate and reacted with an agent and
2 washed. Bound entities are detected.

3

4 The invention also contemplates the use of
5 competitive drug screening assays in which
6 neutralising antibodies such as 791T/36 capable of
7 binding SCR1 and 2 of CD55 specifically compete with
8 a test compound for binding to SCR1 and 2 of CD55.

9

10 Agents identified by the screening method of the
11 present invention and their use in the manufacture
12 of a medicament for the treatment of cancer are also
13 contemplated by the invention.

14

15 Preferred features of each aspect of the invention
16 are as for each of the other aspects *mutatis*
17 *mutandis*.

18

19 The invention will now be described further in the
20 following non-limiting examples. Reference is made
21 to the accompanying drawings in which:

22

23 Figure 1a represents the translated CDR sequences of
24 VK and VH cDNAs from 105AD7 hybridoma. Uppercase
25 letters represent the CDR regions, the lower case
26 letters are the adjacent framework amino acids.

27

28 Figure 1b shows alignment of the three CDR peptides
29 with CD55. The amino acid numbering is taken from
30 the full-length sequence of CD55 including the
31 leader sequence. CD55 peptides used in subsequent
32 assays are shown underlined. Bullets (•) represent

1 amino acid identity whereas amino acids with similar
2 physicochemical properties are marked as (|).

3
4 Figure 2 illustrates a C3b complement deposition
5 assay. 791T cells were incubated with human serum as
6 a source of complement. C3b deposition was measured
7 using rabbit anti-C3b FITC labelled antibody in the
8 presence of blocking (216), non blocking (220) or
9 test antibody 791T/36. Fluorescence was quantified
10 by a FACScan flow cytometer and is present as mean
11 linear fluorescence (MLF).

12 Example 1 CD55 Neutralisation Assay

13
14 Purified CD55 antigen was obtained by
15 immunoaffinity-matrix purification from octyl-
16 glucoside-solubilised 791T cells. CD55 cDNA was
17 cloned and sequenced using primers based on protein
18 sequence data obtained from the purified antigen
19 (Spendlove et al., 1999 Cancer Res 59, 2282). The
20 DNA sequence obtained was identical to that
21 identified by Caras et al and present on the Genbank
22 database (Accession No. M31516).

23 24 **Cells**

25
26 791T is an osteosarcoma cell line which was grown in
27 RPMI (Gibco, BRL, Paisley, and UK) supplemented with
28 10% heat inactivated fetal calf serum.

29 30 **Monoclonal Antibodies**

31

1 Monoclonal antibodies 791T/36 (IgG2b anti-791Tgp72;
2 Embleton et al 1981Br.J. Cancer 43:582-587), BRIC
3 216 (IgG1 anti-SCR 3 of CD55; Tate et al 1989
4 Biochem J 261, 489), BRIC 220 (IgG1 anti-SCR 1 of
5 CD55, Tate et al 1989 Biochem J 261, 489), BRIC 110
6 (IgG1 anti-SCR 2 of CD55; Spring et al., 1987
7 Immunology 62 377; Coyne et al, 1992 J Immunol 149,
8 2906) have been reported previously. The BRIC
9 antibodies were purchased from the Blood Group
10 Reference laboratory (Bristol, UK).

11

12

13 **Methods**

14

15 791T tumour cells that over-express CD55 were washed
16 with media containing 10% FCS and resuspended at a
17 density of 1×10^5 cells per 100 μ l. Primary antibody
18 was incubated with 3x sample volume (3×10^5
19 cells/300 μ l) at a concentration of 50 μ g/ml. Primary
20 antibodies were positive control antibody, 216
21 (anti-SCR3), negative control antibody 220 (anti-
22 SCR1) and test antibody, 791T/36 (anti-SCR1 and 2).
23 Cells and antibodies were incubated for 1 hr at 4°C
24 prior to washing in PBS. Samples were split into 3
25 samples of 100 μ l per tube. Human Serum was added as
26 a source of complement to total concentration of 5%
27 (Not Heat Inactivated). Tubes were inverted several
28 times and incubate at 37°C for 2 hours, mixing every
29 30 min. Cells were washed twice in PBS prior to
30 addition of polyclonal rabbit anti human C3c FITC
31 conjugated antibody (1/100) to a final volume of
32 100 μ l. Cells were incubated for 1 hour at 4°C prior

1 to washing twice in PBS and resuspending in 200µl of
2 1% cell fix.

3

4 **Results**

5

6 Figure 2 shows that in the presence of a non-
7 blocking antibody 220 C3b is deposited onto 791T
8 cells at modest levels (MLF 200). In the presence of
9 the CD55 neutralising antibody, 216, enhanced C3b
10 deposition is observed (MLF 350). However in the
11 presence of monoclonal antibody 791T/36 even greater
12 levels of C3b are deposited (MLF520). This suggests
13 that although 216 is an effective competitor with C3
14 convertase for binding to SCR3. binding of 791T/36
15 to SCR1 and SCR2 domains functionally inactivates
16 CD55 leading to a 250% increase in C3b deposition.

17

18 **Example 2. Long term survival of recurrent**
19 **colorectal cancer patients receiving radiolabelled**
20 **791T/36 for tumour imaging.**

21

22 **Antibody and Labelling**

23

24 Hybridoma 791T/36 clone 3 is the source of antibody
25 (791T/36, IgG2b isotype). Ascitic fluid from mice
26 in which the hybridoma was developing was applied to
27 a protein A-"Sephadex" column in pH 7.5 0.1 mol/l
28 citrate phosphate buffer and the column was
29 thoroughly washed. Bound immunoglobulins were
30 eluted stepwise at pH 6.0, 5.0, 4.5 and 3.0 and
31 these were then dialysed against phosphate-buffered
32 saline. The dialysate was then centrifuged at

1 1000000g for 1 h, filtered through a 0.22µm Millex
2 "Millipore" filter, and stored at -70°C at a protein
3 concentration of 1mg/ml. The preparation contained
4 only IgG2b as assessed by immunodiffusion tests with
5 mouse immunoglobulin typing antisera (Miles
6 Laboratories, Stoke Poges, Bucks.) and was pyrogen-
7 free (Boots Pharmaceuticals, Notts).

8
9 Batches of the antibody preparation were labelled
10 with ¹³¹I by means of "Iodogen" reagent. Non-bound
11 iodine was removed by gel filtration on sephadex
12 G25. Labelled preparations were diluted into saline
13 containing 1% serum albumin and sterilised by Millex
14 filtration.

15
16 72 patients with recurrent colorectal cancer were
17 imaged with the radiolabelled monoclonal antibody
18 791T/36. Patients received an id dose of 10µg of
19 antibody followed by an intravenous dose of 200µg.
20 2dl of preparation containing 200µg of antibody and
21 approximately 70MBq ¹³¹I was infused into an
22 antecubital vein of each patient over 30 min.

23
24 Survival was followed for 7 years and compared to a
25 contemporary group of recurrent colorectal cancer
26 patients. There were 12 long term survivors (16%)
27 in the patients who had received 791T/36 where as in
28 contrast only 1 out of 89 patients survived 7 years
29 in the contemporary group (p> 0.001).

30
31 Table 1: Survival of colorectal cancer patients
32 receiving 791T/36 antibody.

1

Patients	Survival	Death
Imaged with 791T/36	12	60
Contemporary controls	1	88

2

3 These results suggest that there is an apparent
4 survival benefit in a non-randomised trial of
5 patients receiving radiolabelled 791T/36 antibody.
6 The dose of radiolabel reaching the tumour is well
7 below the level required to elicit tumour killing as
8 a result of the radiolabel alone. It is therefore
9 more likely that the antibody is inactivating CD55,
10 allowing complement attack of residual tumour. As
11 these patients only received a single intravenous
12 dose of 791T/36 antibody the apparent survival
13 benefit is very dramatic. Repeat injection with a
14 humanised 791T/36 antibody may have an even more
15 pronounced therapeutic benefit.

16

17 **Example 3. Production of new monoclonal antibodies**
18 **to SCR1 and SCR2**

19

20 6-8 week old Balb/c mice were immunised twice 3
21 weeks apart by intraperitoneal injection with 791T
22 cells that over-express CD55 antigen (10^6 cells).
23 Mice were then boosted with SCR1-2 protein fused to
24 human Fc and purified by protein A chromatography.
25 Mice were tail bled and serum was screened for their
26 ability to recognise CD55SCR1-2/CD46SCR3-4 chimeric
27 molecules expressed by CHO cells as previously
28 described (Spendlove et al 2000 Eur J Immunol 30,
29 2944). They were also screened for their ability to

1 recognise the SCR1-2CD55Fc protein and the IC, 2N
2 and 2C peptides attached to BSA as previously
3 described (Spendlove et al 2000 Eur J Immunol 30,
4 2944). Mice producing antibodies that recognises
5 CD55SCR1 and SCR2 are boosted by an intravenous
6 injection of SCR1-2Fc protein and
7 splenocytes removed 5 days later and fused using PEG
8 with NSO myeloma cells at a 10:1 ratio. Hybridomas
9 are selected using HAT medium and screened for
10 production of antibodies recognising SRR1-2Fc
11 protein by ELISA. Hybridomas producing the correct
12 antibody are cloned by limiting dilution three times
13 a 1 cells per well to ensure clonality. The
14 monoclonal antibody is screened for its ability to
15 recognise CD55SCR1-2/CD46SCR3-4 chimaeric molecules
16 expressed by CHO cells as previously described
17 (Spendlove et al 2000 Eur J Immunol 30, 2944). They
18 are also screened for their ability to recognise the
19 SCR1-2CD55Fc protein and the IC, 2N and 2C peptides
20 attached to BSA as previously described (Spendlove
21 et al 2000 Eur J Immunol 30, 2944). To determine if
22 they recognise the same site as 791T/36 plates are
23 coated with CD55 as described above. They are then
24 incubated with the new monoclonal antibodies and
25 then with biotinylated 791T/36. Binding of 791T/36
26 is quantified by avidin peroxidase and ABTS
27 substrate and the OD read at 405nm on a plate
28 reader. If the monoclonal antibodies recognise the
29 same or related sites to 791T/36 they will inhibit
30 binding of 791T/36 to CD55 antigen.

31

1 All documents referred to in this specification are
2 herein incorporated by reference. Various
3 modifications and variations to the described
4 embodiments of the inventions will be apparent to
5 those skilled in the art without departing from the
6 scope and spirit of the invention. Although the
7 invention has been described in connection with
8 specific preferred embodiments, it should be
9 understood that the invention as claimed should not
10 be unduly limited to such specific embodiments.
11 Indeed, various modifications of the described modes
12 of carrying out the invention which are obvious to
13 those skilled in the art are intended to be covered
14 by the present invention.

15

16 References

17

- 18 1. Liszewski, M.K., T.C. Farries, D.M. Lublin,
19 I.A. Rooney, and J.P. Atkinson. 1996. *Adv*
20 *Immunol* 61:201-283.
- 21 2. Hindmarsh, E.J., and R.M. Marks. 1998. *Eur J*
22 *Immunol* 28:1052-1062.
- 23 3. Niehans, G.A., D.L. Cherwitz, N.A. Staley, D.J.
24 Knapp, and A.P. Dalmasso. 1996. *Am J Pathol*
25 149:129-142.
- 26 4. Li, L., I. Spendlove, J. Morgan, and L.G.
27 Durrant. 2001. *Br. J. Cancer* 84:80-86.
- 28 5. Thorsteinsson, L., G.M. O'Dowd, P.M.
29 Harrington, and P.M. Johnson. 1998. *APMIS*
30 106:869-878.

- 1 6. Yamakawa, M., K. Yamada, T. Tsuge, H. Ohrai, T.
2 Ogata, M. Dobashi, and Y. Imai. 1994. *Cancer*
3 73:2808-2817.
- 4 7. Bjorge, L., T.S. Jensen, and R. Matre. 1996.
5 *Cancer Immunol Immunother* 42:185-192.
- 6 8. Simpson, K.L., A. Jones, S. Norman, and C.H.
7 Holmes. 1997. *Am J Pathol* 151:1455-1467.
- 8 9. Juhl, H., F. Helmig, K. Baltzer, H. Kalthoff,
9 D. HenneBruns, and B. Kremer. 1997. *J. Surgical*
10 *Oncol.* 64:222-230.
- 11 10. Hindmarsh, E.J., and R.M. Marks. 1998. *J.*
12 *Immunol.* 160:6128-6136.
- 13 11. Niehans, G.A., D.L. Cherwitz, N.A. Staley, D.J.
14 Knapp, and A.P. Dalmasso. 1996. *Am. J. Pathol.*
15 149:129-142.
- 16 12. Azuma, A., Y. Yamano, A. Yoshimura, T. Hibino,
17 T. Nishida, H. Yagita, K. Okumura, T. Seya, R.
18 Kannagi, M. Shibuya, and S. Kudoh. 1995. *Scand*
19 *J Immunol* 42:202-208.
- 20 13. Blok, V.T., M.R. Daha, O. Tijssma, C.L. Harris,
21 B.P. Morgan, G.J. Fleuren, and A. Gorter. 1998.
22 *J Immunol* 160:3437-3443.
- 23 14. Harris, C.L., K.S. Kan, G.T. Stevenson, and
24 B.P. Morgan. 1997. *Clin. Exp. Immunol.* 107:364-
25 371.
- 26 15. Farrands, P.A., A.C. Perkins, M.V. Pimm, J.D.
27 Hardy, M.J. Embleton, R.W. Baldwin, and J.D.
28 Hardcastle. 1982. *Lancet* 2:397-400.
- 29 16. Farrands, P.A., A. Perkins, L. Sully, J.S.
30 Hopkins, M.V. Pimm, R.W. Baldwin, and J.D.
31 Hardcastle. 1983. *J. of Bone and Joint Surg.*
32 65:638-640.

- 1 17. Armitage, N.C., A.C. Perkins, M.V. Pimm, M.L.
2 Wastie, and R.W. Baldwin. 1985 *Nucl Med Commun*
3 6:623-631.
- 4 18. Armitage, N.C., A.C. Perkins, M.V. Pimm, P.A.
5 Farrands, R.W. Baldwin, and J.D. Hardcastle.
6 1984. *Br J Surg* 71:407-412.
- 7 19. Byers, V.S., M.V. Pimm, P.J. Scannon, I.
8 Pawluczyk, and R.W. Baldwin. 1987. *Cancer Res*
9 47:5042-5046.
- 10 20. Byers, V.S., R. Rodvien, K. Grant, L.G.
11 Durrant, K.H. Hudson, R.W. Baldwin, and P.J.
12 Scannon. 1989. *Cancer Research* 49:6153-6160.
- 13 21. Spendlove, I., L. Li, J. Carmichael, and L.G.
14 Durrant. 1999. *Cancer Res.* 59:2282-2286.
- 15 22. Embleton, M.J., B. Gunn, V.S. Byers, and R.W.
16 Baldwin. 1981. *Br.J. Cancer* 43:582-587.
- 17 23. Loveland, B.E., M. Lanteri, P. Kyriakou, and D.
18 Christiansen. 1998. *Molecular Immunology* 35:369
19 A155.
- 20 24. Lanteri, M., D. Christiansen, P.M. Hogarth,
21 I.F.C. McKenzie, and B.E. Loveland. 1998.
22 *Molecular Immunology* 35:369 A156.
- 23 25. Evans, M.J., S.L. Hartman, D.W. Wolff, S.A.
24 Rollins, and S.P. Squinto. 1995 *J. Immunol.*
25 *Methods* 184:123-138.
- 26 26. Casasnovas, J.M., M. Larvie, and T. Stehle.
27 1999. *The EMBO J.* 18:2911-2922.
- 28 27. Friedman, A.R., V.A. Roberts, and J.A. Tainer.
29 1994. *Proteins: Struct. Funct. Genet.* 20:15-24.
- 30 28. Stanfield, R.L., M. Takimoto-Kamimura, J.M.
31 Rini, A.T. Profy, and I.A. Wilson. 1993.
32 *Structure* 1:83-93.

- 1 29. Coyne KE, Hall SE, Thompson ES Arce MA,
2 Inoshita T, Fujita T, Anstee DJ, Rosse W,
3 Lublin DM (1992). J Immunol 149 2906-2913.
- 4 30. Spring FA, Judson PA, Daniels SF, Parsons SF,
5 Mallinson G and Anstee DJ (1987). Immunology 62
6 377.
- 7 31. Tate CG, Uchikawa M, Tanner MJA, Judson PA,
8 Parsons SF, Mallinson G and Anstee DJ (1989).
9 Biochem J 261, 489.
- 10 32. Gorter a, Blok VT, Haasnoot WHB, Ensink NG,
11 Daha MR, Leuren GJ (1996) Lab Invest 74 1039-
12 1049.
- 13 33. Spendlove I, Li L, Potter V, Christiansen D ,
14 Loveland B and Durrant LG (2000) Eur J Immunol
15 30, 2944.
- 16
- 17